PNPase knockout results in mtDNA loss and an altered metabolic gene expression program.

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Public Summary:
Polynucleotide phosphorylase (PNPase) is an essential mitochondria-localized exoribonuclease implicated in multiple biological processes and human disorders. To reveal role(s) for PNPase in mitochondria, we established PNPase knockout (PKO) systems by first shifting culture conditions to enable cell growth with defective respiration. Combined, our study reports that knockout of a mitochondrial nuclease results in mtDNA loss and suggests that mtDNA maintenance could provide a unifying connection for the large number of biological activities reported for PNPase. This study supports continued investigation in the maintenance of the mitochondrial DNA genome and its importance in stem cell maintenance.

Scientific Abstract:
Polynucleotide phosphorylase (PNPase) is an essential mitochondria-localized exoribonuclease implicated in multiple biological processes and human disorders. To reveal role(s) for PNPase in mitochondria, we established PNPase knockout (PKO) systems by first shifting culture conditions to enable cell growth with defective respiration. Interestingly, PKO established in mouse embryonic fibroblasts (MEFs) resulted in the loss of mitochondrial DNA (mtDNA). The transcriptional profile of PKO cells was similar to rho0 mtDNA deleted cells, with perturbations in cholesterol (FDR = 6.35 x 10-13), lipid (FDR = 3.21 x 10-11), and secondary alcohol (FDR = 1.04x10-12) metabolic pathway gene expression compared to wild type parental (TM6) MEFs. Transcriptome analysis indicates processes related to axonogenesis (FDR = 4.49 x 10-3), axon development (FDR = 4.74 x 10-3), and axonal guidance (FDR = 4.74 x 10-3) were overrepresented in PKO cells, consistent with previous studies detailing causative PNPase mutations in delayed myelination, hearing loss, encephalomyopathy, and chorioretinal defects in humans. Overrepresentation analysis revealed alterations in metabolic pathways in both PKO and rho0 cells. Therefore, we assessed the correlation of genes implicated in cell cycle progression and total metabolism and observed a strong positive correlation between PKO cells and rho0 MEFs compared to TM6 MEFs. We quantified the normalized biomass accumulation rate of PKO clones at 1.7% (SD +/- 2.0%) and 2.4% (SD +/- 1.6%) per hour, which was lower than TM6 cells at 3.3% (SD +/- 3.5%) per hour. Furthermore, PKO in mouse inner ear hair cells caused progressive hearing loss that parallels human familial hearing loss previously linked to mutations in PNPase. Combined, our study reports that knockout of a mitochondrial nuclease results in mtDNA loss and suggests that mtDNA maintenance could provide a unifying connection for the large number of biological activities reported for PNPase.